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TITLE: The Effect of Hypotensive Resuscitation and Fluid Type on Mortality, Bleeding, Coagulation and Dysfunctional Inflammation in a Swine Grade V Liver Injury Model

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14. ABSTRACT Objectives: To determine the efficacy and safety of lyophilized plasma for the treatment of multisystem trauma in pigs. Methods: 1. 32 swine at 2 institutions underwent femur fracture, controlled hemorrhage (30% estimated blood volume), hypothermia (33°C) and resuscitation with normal saline and Grade V liver injury followed by 30 minutes of hemorrhagic shock without resuscitation. Animals were then randomized to Fresh Frozen Plasma (FFP), Lyophilized Plasma (LP), 1:1 PRBC:FFP, and 1:1 PRBC:LP. Animals were followed for four hours post liver injury. Physiologic parameters, coagulation assays and inflammatory mediators were compared. Results: 1. There were no significant differences in any physiologic parameters (Mean Arterial Pressure, Heart Rate, Mortality, or Blood Loss) between the groups. Coagulation parameters did not differ between groups. Analysis of inflammatory markers showed significantly lower values for LP animals compared to FFP animals at 2 hours post injury. Conclusion: LP is as safe and effective as FFP for resuscitation in a severe hemorrhagic shock model and it may reduce harmful inflammation associated with severe trauma.						
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INTRODUCTION:

Exsanguination is the leading cause of death on the battlefield. Lifesaving interventions include arresting hemorrhage and initiating resuscitation. The ideal resuscitation of combat casualties has not been determined. The traditional goal of resuscitation has been to restore perfusion. There is increasing evidence to suggest that early correction of coagulopathy improves mortality. Transfusion of a 1:1 ratio of plasma to packed red blood cells has been shown to result in improved survival compared to lower ratios. However, due to the need to freeze plasma and its limited availability in austere setting, it is difficult to achieve these ratios in combat scenarios. During this past year we addressed two more of the original eight specific aims from the scope of work. The purpose of this specific aim 5 was to determine the efficacy and safety of lyophilized plasma in complex multi-system animal model that we have previously developed. Our first objective was to determine the effect of the lyophilization process on coagulation factor function by evaluating the in-vitro clotting ability of plasma before lyophilization and after reconstitution. The work from specific aim 4 was to compare the efficacy of LP to FFP in the resuscitation of pigs undergoing severe multisystem trauma. This work was performed at 2 institutions to insure the results were reproducible.

BODY:

Materials and Methods

Specific Aim 5 – Determination of the Effect of Lyophilization on Coagulation Factor Activity

Yorkshire crossbred donor swine were anesthetized and underwent blood typing. The left ventral cervical area was steriley prepped and the left external jugular vein was cannulated. Animals were then exsanguinated utilizing a centrifugal pump. Blood was collected in citrated Terumo Teruflex triple blood donation bags, centrifuged at 5000g for 9 minutes at 4 degrees centigrade. Plasma was removed using a Baxter Plasma Extractor. Plasma was frozen and stored at -20 degrees centigrade until collected by HemCon for creation of the lyophilized product.

Specific Aim 4 - Hemostatic Resuscitation in a Multi-system Combat Relevant Model

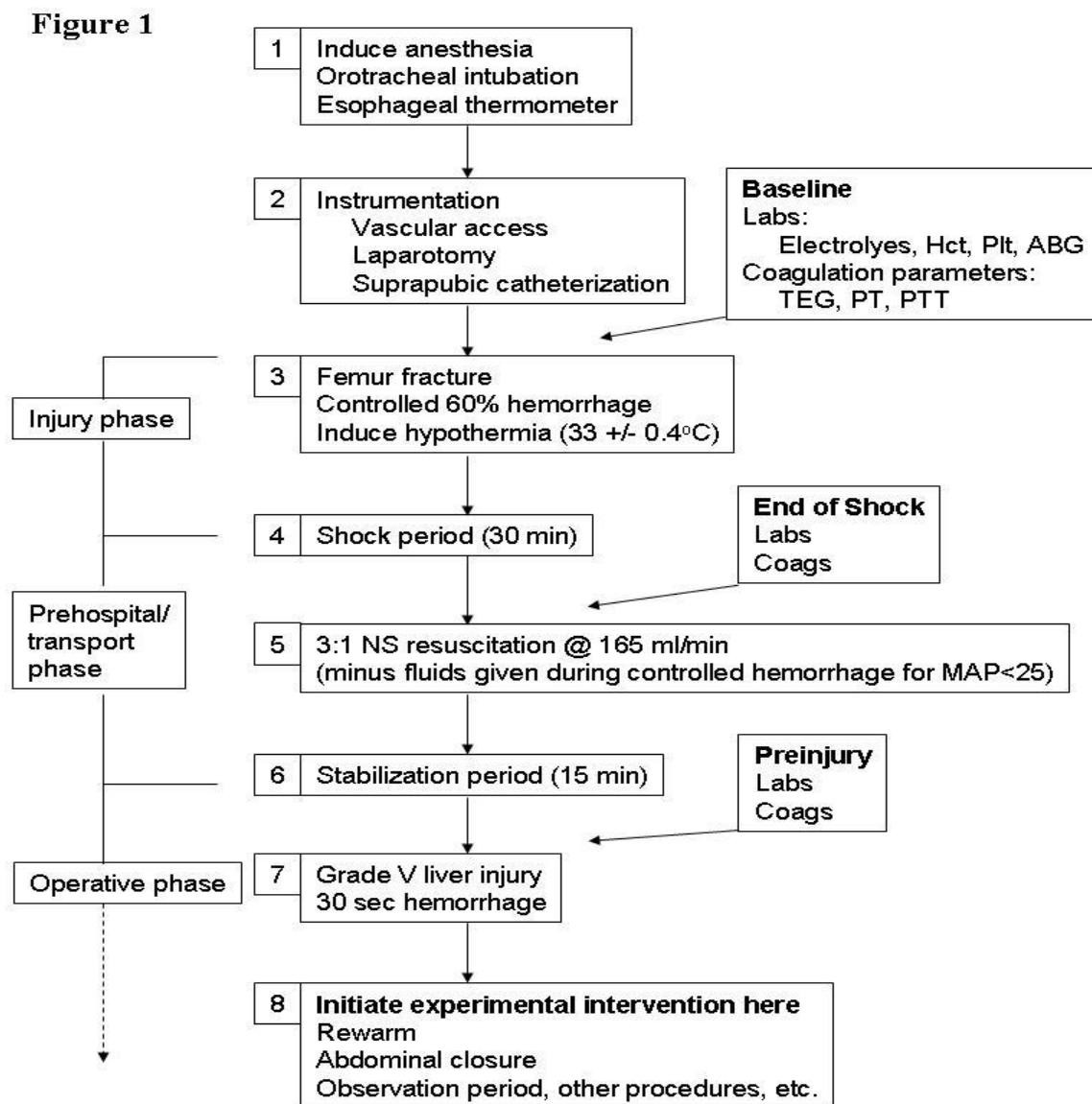
The model was developed at the Oregon Health & Science University (OHSU), and shared with the United States Army Institute of Surgical Research (USAISR). This process was described in the yearly report from 2007.

We developed a complex, combat-relevant, multisystem injury model of liver injury, long bone fracture and soft tissue injury and hemorrhagic shock with hypothermia and acidosis. We simulated an injury phase, a preoperative phase (including prehospital care, transport and emergency department care) and an operative phase of resuscitation. (Figure 1)

Study protocol

Thirty-two female Yorkshire crossbred swine were utilized. Animals were delivered 7-10 days prior to the experiment in order to minimize the stress of transport and subsequent potential changes in sympathetic output or inflammatory mediators. An overnight fasting period was observed with the exception of water ad libitum. All animals were ordered such that their weight at the time of the experiment was 36.4 ± 0.7 kg (mean \pm SEM). No attempt was made to use a single vendor, and each center made their own arrangements for procurement of animals according to their standard sources. In addition to the 32 animals that were used for the study protocol, an additional 6 were used in model development and 28 for creation of the donor plasma/prbc's.

Figure 1



Anesthesia

Anesthesia was induced with 8 mg/kg Telazol® (tiletamine hydrochloride 50 mg/ml, zolazepam hydrochloride 50 mg/ml, Fort Dodge Animal Health, Fort Dodge, Iowa) intramuscularly and isoflurane at 1-3% inhaled. Orotracheal intubation was performed after which an esophageal thermometer was placed. Throughout the study anesthesia was maintained to the clinical endpoints of reflexes and muscle relaxation as is done in humans.

Monitoring, access and pre-experiment procedures

Vascular access was established via neck cutdown and placement of carotid artery and external and internal jugular vein catheters. The femoral artery was cannulated for blood pressure monitoring. Baseline labs were collected and included electrolytes, lactate, spun hematocrit (Hct), activated clotting time (ACT), platelets (Plt), prothrombin time (PT), partial thromboplastin time (PTT), and arterial blood gas (ABG). In addition, a baseline thrombelastogram (TEG, Haemoscope Corporation, Niles, IL) was performed. A celiotomy was then performed, at which time a suprapubic bladder catheter was placed to monitor urine output.

Injury phase

After needle localization, a captive bolt gun was used to fracture the femur and create a soft tissue injury at the midshaft of the left femur. Figure 2 is a 3-D computed tomography (CT) reconstruction of a typical femur fracture created in a study animal by these methods. A controlled hemorrhage was then initiated to remove 60% of the blood volume based on a published, standard equation relating blood volume to body weight for domestic swine. During this period if the mean arterial blood pressure (MAP) fell below 25mm/Hg, normal saline (NS) was infused at a rate of 165 ml/min to keep the MAP>25 mm/Hg. The animal was then cooled to $33 \pm 0.4^{\circ}\text{C}$ using cooled intraperitoneal lavage with crystalloid as needed (most of the animals developed a degree of hypothermia spontaneously due to shock and infusion of IV fluids). These procedures were followed by a 30-minute shock period, representing time in the field prior to medical intervention.

Prehospital care/transport phase

After the 30-minute shock period, electrolytes, spun hematocrit, ACT, PT, PTT, platelets, ABG, and TEG were again recorded. After coagulation studies and lab collection, the hemorrhage volume was replaced with a 3:1 ratio of NS infused at a rate of 165 ml/min, minus any given during the controlled hemorrhage. This reflects current civilian prehospital resuscitative practices.

Operative phase

Following NS resuscitation, a 15-minute stabilization period was observed, during which a baseline MAP was recorded and preweighed laparotomy sponges were placed in both paracolic

gutters and in the pelvis for blood collection. Labs and coagulation studies were again collected, and a previously described grade V liver injury was created at the confluence of the right and middle hepatic veins using a specialized clamp.

Figure 2. CT scan showing extent of the femur fracture. The arrow points to the fractured left femur.



Thirty seconds of hemorrhage were then followed by evacuation of blood from the abdomen and packing of the liver with a fixed number of additional preweighed laparotomy sponges. The liver injury was designed to provide a second stressor after initial injury and also to create a standardized injury that had the potential to rebleed, both of which simulate a laparotomy after trauma in a patient with solid organ injury. Thirty seconds after injury, the liver was packed with laparotomy sponges in a standardized fashion. Randomized treatments were initiated at the same time as packing was initiated. Randomization groups included FFP, LP, 1:1 FFP:PRBCs, and 1:1 LP:PRBCs. The 1:1 groups were utilized because transfusion of FFP and PRBCs in a ration of 1:1 is the currently recommended standard in combat victims undergoing massive transfusion protocols. The volume of the treatment resuscitation was equivalent to the blood loss from the controlled hemorrhage.

Study Variables

Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss due to the liver injury. Laboratory values include Hct, lactate, Plt, ABG, and electrolytes. Coagulation parameters include the PT, PTT, and ACT.

Statistical Analysis

Mean values of study variables between centers were compared using a one-way analysis of variance (ANOVA). We assumed that our study populations were normally distributed and that the variances of the populations were equal. A post-hoc Bonferroni correction was applied to account for multiple comparisons. The significance level was set at a p value of less than 0.05. Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, Illinois).

Serum and Tissue Cytokine Analysis

Serum cytokine levels were quantified using the Quantikine® enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN).

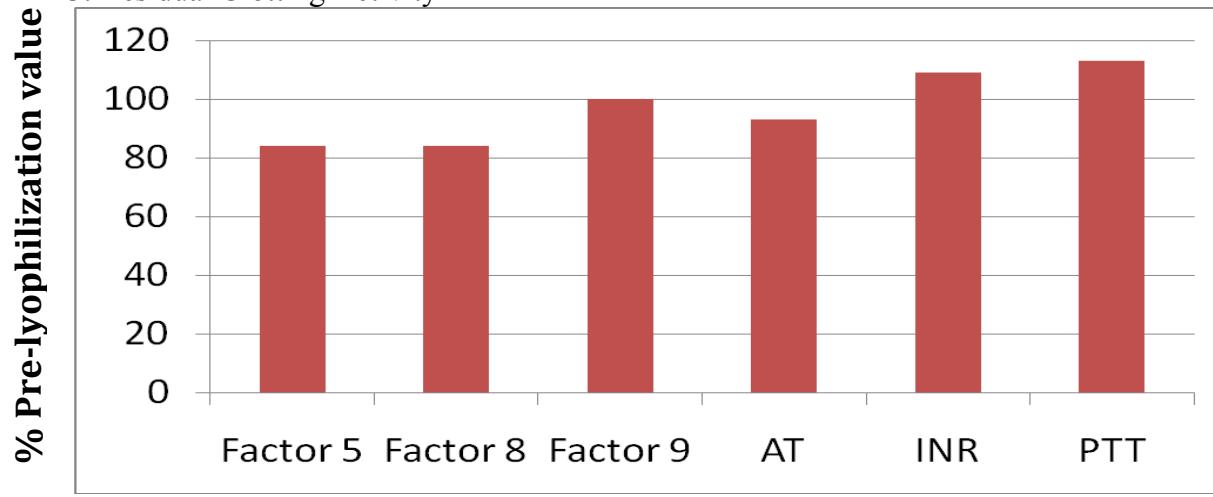
RESULTS

Specific Aim 5

In Vitro Analysis

Factor levels and clotting parameters were measured before lyophilization and after reconstitution. Compared to the plasma before lyophilization, there was a drop in the clotting activity by an average of 14%. This compares favorably with fresh frozen human plasma that retains only 60-70% of its original clotting factor activity. A comparison of the percent of the pre-lyophilization factor values (for industry recognized standards) is shown in Figure 3. Also included in Figure 3 is percent increase in INR and PTT compared to pre-lyophilization values.

Figure 3. Residual Clotting Activity



Specific Aim 4

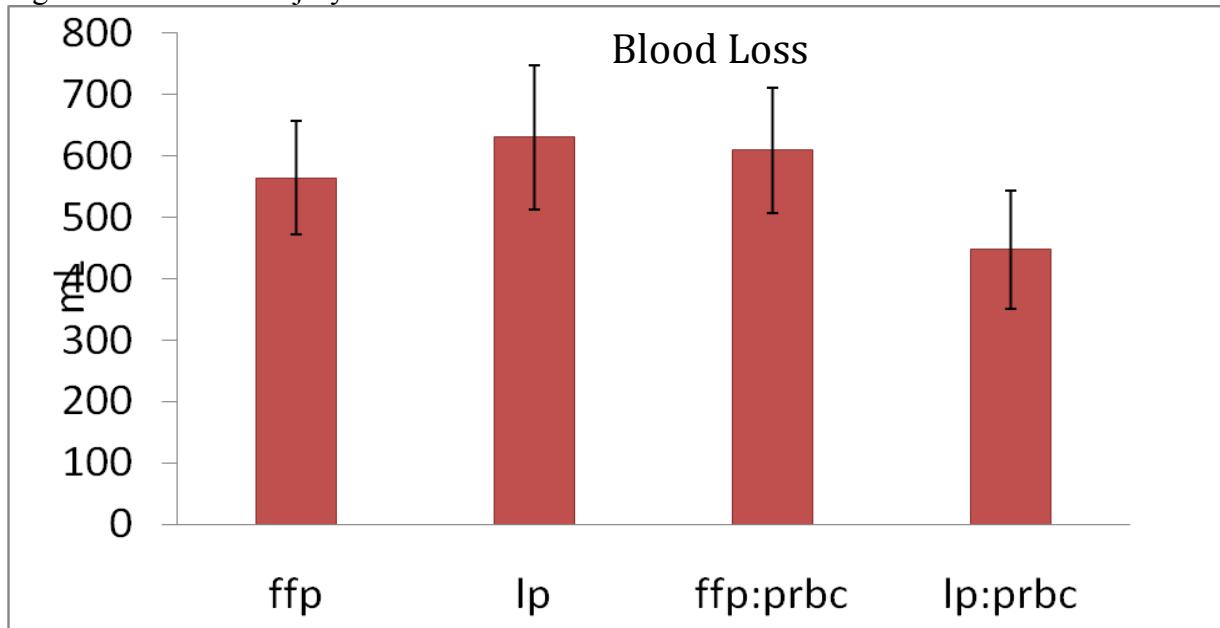
Results of randomized resuscitation

Physiologic variables

Hypothermia was achieved during the shock period, with a pre liver injury temperature of $32.9 \pm 0.06^\circ\text{C}$. Blood loss from the controlled hemorrhage, a function of the calculated blood volume, was $1602.7 \pm 23.5 \text{ ml}$ or $44.3 \pm 0.3 \text{ ml/kg body weight}$.

No significant differences were seen between the groups with respect to blood loss following liver injury. (Figure 4).

Figure 4. Post Liver Injury Blood Loss



Prior to randomization animals in all injury groups had a similar physiologic profile with an acute drop in blood pressure, followed by auto-resuscitation. Post injury mean arterial pressures (MAP) and Heart Rates across groups are shown in Figure 5 and 6. Neither MAP nor HR differed significantly between animals in the injury groups at the end of the study. Standard error bars reveal minimal variation between animals within groups.

Figure 5. Post Injury Mean Arterial Blood Pressures across Groups

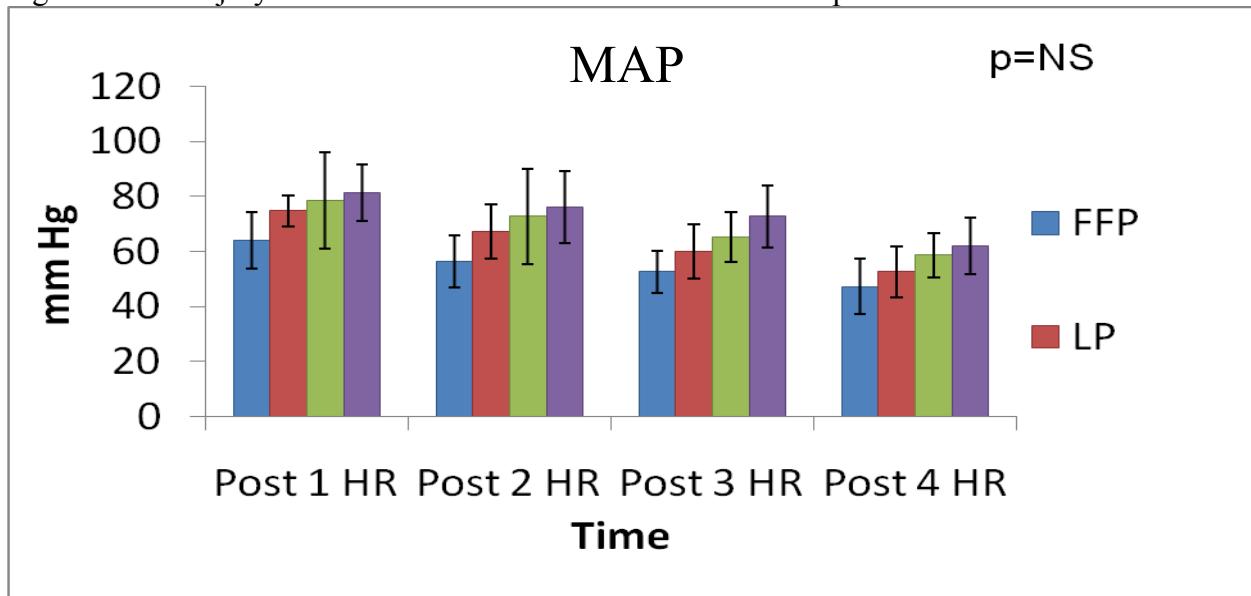
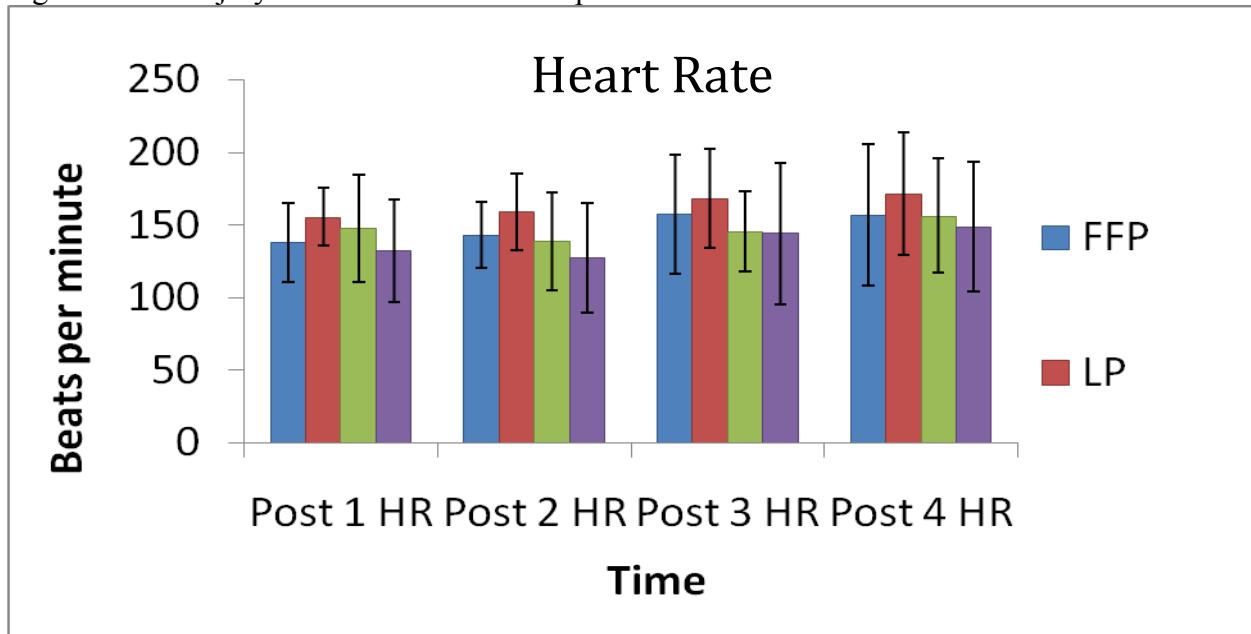


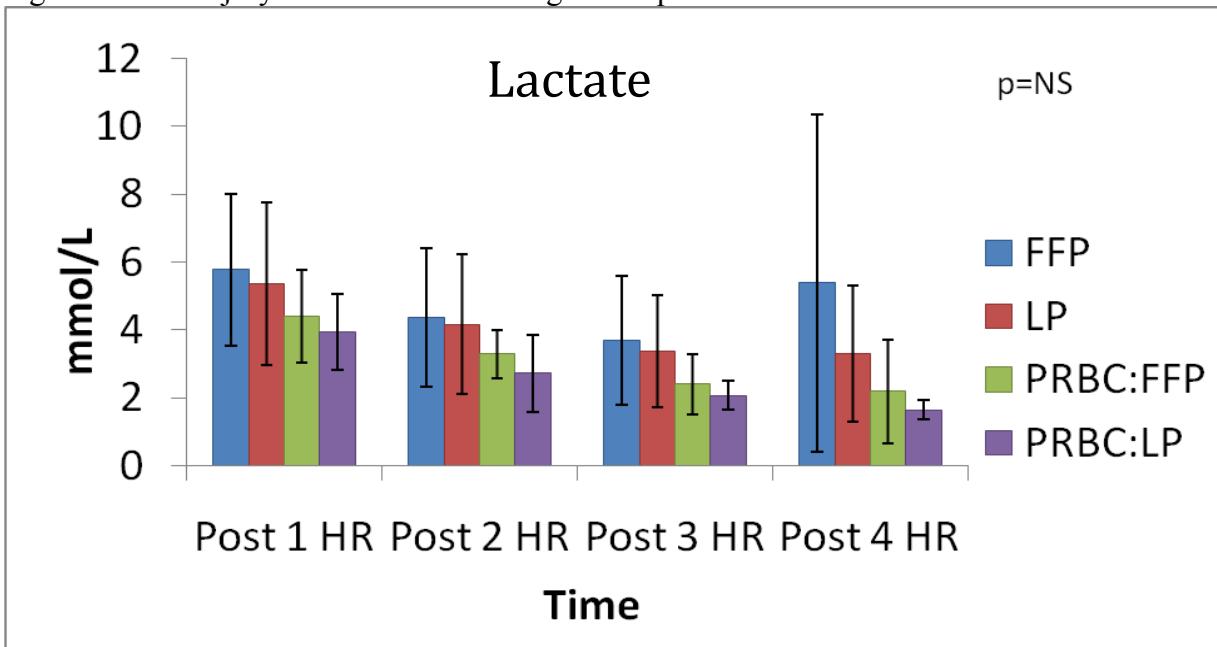
Figure 6. Post Injury Heart Rate across Groups



Laboratory variables

No statistical difference was noted between any of the groups with respect to the laboratory values (lactate, platelets, ABG, and electrolytes) that were collected. Figure 7 shows a similar progressive improvement in lactate levels among all groups with the exception of FFP.

Figure 7. Post Injury Lactate Levels amongst Groups



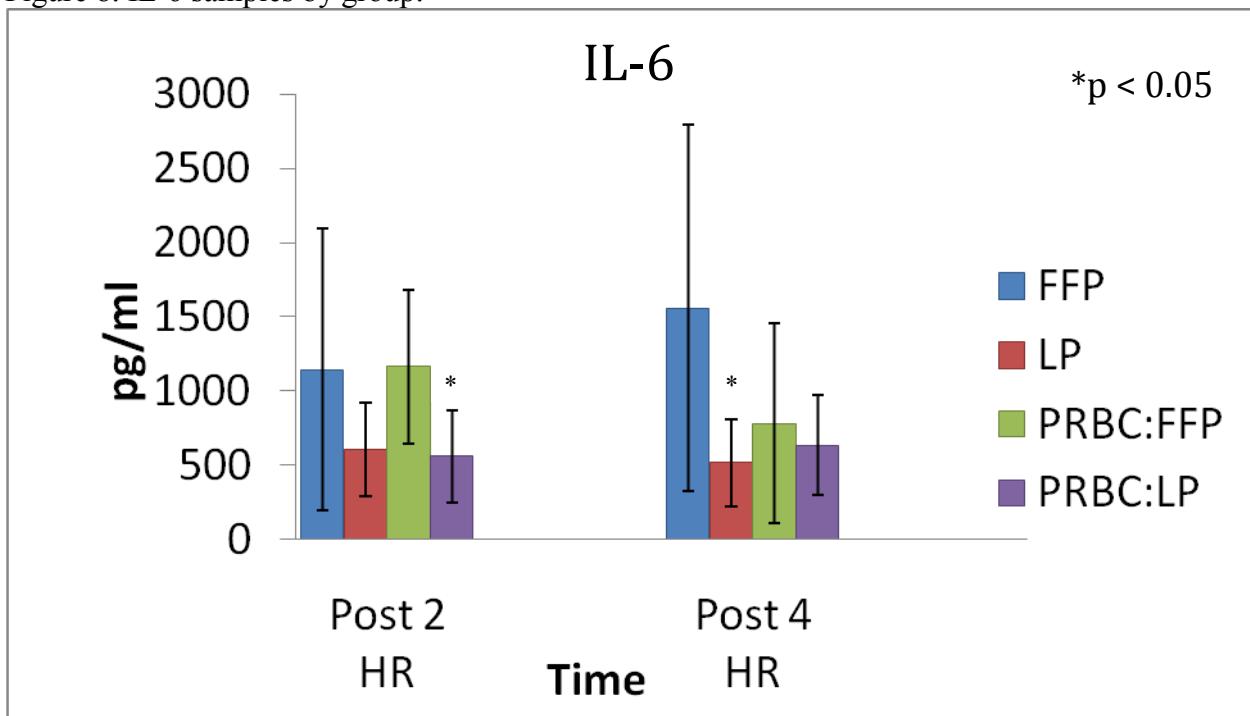
Coagulation parameters

Activated Clotting Time, PT and PTT values were measured in all four groups at baseline and hourly for four hours after study fluid administration. Importantly, there were no differences between LP and FFP or between the 1:1 ratio groups with respect to coagulation measurements at any time.

Inflammatory Parameters

Serum samples were analyzed for markers of inflammation that included IL-6, IL-8, and TNF- α . Prior to injury, there were no differences seen between groups. Swine randomized to the FFP group had significant increases in IL-6 at both 2 and 4 hours post injury in comparison to their baseline values. In addition, TNF- α in the FFP group showed a significant increase at the 4 hour mark. A significant decrease in IL-8 production was found at the 4 hour mark in the FFP group. In swine resuscitated with 1:1 FFP:PRBC; IL-6, IL-8 and TNF- α were increased at 2h, but only TNF- α remained elevated at 4h. 1:1 LP:PRBC resuscitated swine had increased IL-8 at 2h only. In regards to the LP samples, no significant differences were seen in either IL-8 or TNF- α when compared to baseline. Compared to FFP, IL-6 levels in the LP group were significantly less at the 4 hour mark (Figure 8). Overall, LP resuscitated pigs had equivalent or less inflammation than FFP pigs.

Figure 8. IL-6 samples by group.



KEY RESEARCH ACCOMPLISHMENTS

1. A severe multi-system combat relevant shock model can be reproduced at multiple centers.
2. The clotting activity of lyophilized plasma is favorable in comparison to FFP.
3. Resuscitation with LP results in similar hemodynamic and physiologic outcomes in comparison to FFP.
4. Resuscitation with LP, FFP and 1:1 PRBCs:FFP and 1:1 PRBCs:LP results in similar mortality and similar end of study coagulation parameters.
5. Resuscitation with LP results in less expression of IL-6 compared to FFP or 1:1 ratios at 2 HR post injury.
6. Lyophilized plasma may be superior to FFP for the resuscitation of massive trauma based on superior logistical requirements, superior retention of clotting factor function and decreased dysfunctional inflammation.

REPORTABLE OUTCOMES

This work was presented and won the 2008 Region X Residents' Basic Science Competition of the American College of Surgeons Committee on Trauma.

The physiologic and coagulation components of this work are scheduled to be presented at the Pacific Coast Surgical Association meeting in February 2009.

The inflammatory component of this work is scheduled to be presented at the 2009 combined meeting of the Association of Academic Surgeons and the Society of University Surgeons.

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